PRIMER FOR DETECTION OF HUMAN PAPILLOMAVIRUS

Technical Field

The present invention relates to primers specific to the genome of human papillomavirus (hereinafter, referred to as "HPV"), a kit for detecting the HPV genome comprising the primers, and a method of detecting the HPV genome using the primers.

Background Art

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HPV is a double-stranded DNA virus whose circular genome is approximately 8 kb long. HPV inhabits the vagina, and the infection thereof is hard to treat and is not easily made to disappear. HPV infects the epithelial cells of other mammals as well as humans, and generally induces warts, and sometimes malignant tumors, at the site of infection. HPV is detected in over 90% of condyloma accuminata cases (enlarged warts having a cauliflower-like appearance around the genitals or the anus) and almost 100% of cervical cancer cases. In particular, cervical cancer accounts for 22.1% of all cancers found in women in Korea, and is the second leading cause of cancer death among women.

Thus, establishing a method of effectively detecting HPV, which causes cervical cancer, is important for the

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diagnosis, prophylaxis and therapy of the disease. Also, HPV needs to be effectively detected to evaluate the efficacy and toxicity of a vaccine against HPV after vaccination.

nucleic acid-based test for diagnosing infectious disease employs a standard method of isolating nucleic acids from individuals and clinical materials. Since target DNA or RNA is present in clinical specimens in small amounts, several major techniques used in diagnostic laboratories are based on signal amplification and target amplification. These methods aid detection, are useful in the identification of individuals without culture, and contribute to the treatment as well as diagnosis of PCR, which is infectious diseases. а nucleic acid amplification technique (NAT), is widely used because it enables the selective amplification of specific targets, present in low concentrations, to detectable levels. addition the qualitative detection of to viruses, quantitative determination of viral load in specimens is now realized to be of great importance with respect to the diagnosis, prognosis, and therapeutic monitoring of HPV infection (Pfaller M.A, Emer. Infect. Dis. 7, 2, 2001).

The genome of all types of HPV is divided into two major regions: early and late regions. The early region of about 4.5 kb codes for genes which are associated with

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functions including viral DNA replication (E1), induction or suppression of the action of DNA encoding a protein inducing malignant transformation of host cells (E2), synthesis of proteins responsible for the growth of host cells and viruses (E4), stimulation of the activity of epidermal growth factor (EGF) and colony stimulator factor (CSF) receptors (E5), and malignant transformation through permanent survival of cells, activation of oncogenes and inactivation of tumor suppressor genes (E7). In particular, the oncogenic E6 and E7 proteins, which are expressed after HPV infects the epithelial cells of a host, bind to tumor suppressor proteins of host cells, p53 and respectively, thereby inhibiting the function of the tumor suppressor proteins, leading to the transformation of infected cells, resulting in the development of tumors. The late region of 2.5 kb comprises genes coding for viral major (L1) and minor (L2) capsid proteins and a non-coding region of 1 kb, which is called the long control region (LCR) that regulates the transcription and translation of the two late genes.

With recent rapid advances in molecular biological techniques, the genetic structure of HPV has been identified, revealing genomic sequences of many genotypes of HPV. HPV is classified according to the difference in DNA sequences of E6, E7 and L1 open reading frames (ORFs). When the nucleotide sequences of the ORFs differ by more

than 10%, an HPV is assigned a new genotype. HPV subtypes differ by 2% to 10%, and HPV variants differ by less than 2%.

In order to specifically detect high risk HPV types 16, 18 and 31 and a low risk HPV type 11 among a large number of HPV genotypes, which are detected in tissues of cervical cancer and carcinoma in situ, respectively, the present inventors intended to detect a gene specific to each genotype of these viruses, and selected the L1 gene as such a gene.

In order to specifically detect the HPV L1 gene, the present inventors determined the sequences of L1 genes of the HPV types 11, 16, 18 and 31, which are specifically found in Koreans, and constructed primers capable of specifically binding to the L1 gene of each HPV type. The present inventors found that when PCR was performed with the primers, each HPV genotype can be specifically detected and can be precisely quantified down to very low amounts, thereby leading to the present invention.

20 Disclosure of the Invention

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It is therefore an object of the present invention to provide a primer pair selected from among pairs of primers capable of complementarily binding to the genome of human papillomavirus (HPV) and having nucleotide sequences

represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.

It is another object of the present invention to provide a method of detecting the HPV genome, which is based on performing a polymerase chain reaction (PCR) for DNA contained in a biological sample using one or more primer pairs selected from among pairs of primers capable of complementarily binding to the HPV genome and having nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.

It is a further object of the present invention to provide a kit for detecting the HPV genome, comprising one or more primer pairs selected from among pairs of primers capable of complementarily binding to the HPV genome and having nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.

Brief Description of the Drawings

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The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

Fig. 1 schematically represents the construction of

recombinant plasmids constructed with amplified L1 genes of HPV genotypes 11, 16, 18 and 31 (A), and also shows the results of restriction enzyme mapping of the recombinant plasmids (B);

Fig. 2 is an alignment of HPV 16 L1 sequences;

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- Fig. 3 is an alignment of HPV 31 L1 sequences;
- Fig. 4 is an alignment of HPV 11 L1 sequences;
- Fig. 5 is an alignment of HPV 18 L1 sequences;
- Fig. 6 is a multiple alignment of nucleotide

 10 sequences of L1 genes of HPV genotypes 11, 16, 18 and 31

 and the consensus L1 sequence;
 - Fig. 7 shows the results of a sensitivity test using plasmid DNA templates, each of which carry the HPV 11, 16, 18 or 31 L1 gene;
- 15 Fig. 8 shows the results of a differentiality test using plasmid DNA templates, each of which carries the HPV 11, 16, 18 or 31 Ll gene;
 - Fig. 9 shows the results of heat stability and longterm preservation tests for HPV L1 plasmids after storage for 3 weeks;
 - Figs. 10 and 11 show the results of heat stability and long-term preservation tests for HPV L1 plasmids after storage for 15 weeks; and
- Fig. 12 shows the results of an applicability test
 using predetermined amounts of HPV L1 plasmids supplemented
 with various DNA backgrounds.

Best Mode for Carrying Out the Invention

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In one aspect, the present invention relates to primers capable of complementarily binding to the HPV genome.

In a detailed aspect, the present invention relates to a primer pair selected from among pairs of primers capable of complementarily binding to the HPV genome and having the nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.

The term "primer", as used herein, refers to a short nucleic acid sequence having a free 3' hydroxyl group, which to undergo base-pairing interaction with a complementary template and serves as a starting point for replicating the template strand: A primer is able to initiate DNA synthesis in the presence of a reagent for polymerization and four different nucleoside triphosphates in suitable buffers and at a suitable temperature. With respect to the objects of the present invention, primers specifically amplify a specific region of the L1 gene of HPV 16, described in Fig. 2, HPV 31, described in Fig. 3, HPV 11, described in Fig. 4, and HPV 18, described in Fig. 5. Thus, the primers of the present invention consist of a pair of sense and antisense primers having a sequence of 7 and more preferably 10 30 50 nucleotides,

nucleotides, the sequence capable of complementarily binding to the aforementioned HPV L1 gene. In detail, a specific region of the HPV L1 gene may be specifically amplified with a pair of primers having the nucleotide sequences of SEQ ID Nos. 1 and 2 for HPV 11, a pair of primers having the nucleotide sequences of SEQ ID Nos. 3 and 4 for HPV 16, a pair of primers having the nucleotide sequences of SEQ ID Nos. 5 and 6 for HPV 18, and a pair of primers having the nucleotide sequences of SEQ ID Nos. 7 and 8 for HPV 31.

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The primers of the present invention may chemically synthesized using a phosphoramidite support method or other widely known methods. These nucleic acid sequences may also be modified using any means known Non-limiting examples of such modifications in the art. include methylation, capsulation, replacement of one or more native nucleotides with analogues thereof, and internucleotide modifications, for example, modifications to conjugates (e.g., methyl phosphonate, uncharged phosphotriester, phosphoroamidate, carbamate, etc.) charged conjugates (e.g., phosphorothioate, phosphorodithioate, etc.). Nucleic acids may contain one or more additionally covalent-bonded residues, which are exemplified by proteins (e.g., nucleases, toxins, signal peptides, poly-L-lysine, antibodies. intercalating agents (e.g., acridine, psoralene,

chelating agents (e.g., metals, radioactive metals, iron, oxidative metals, etc.), and alkylating agents. The nucleic acid sequences of the present invention may also be altered using a label capable of directly or indirectly supplying a detectable signal. Examples of such a label include radioisotopes, fluorescent molecules, and biotin.

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When PCR was performed with the primers provided in the present invention, which have the nucleotide sequences of SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8, the primers were found to be able to specifically detect each of the four different HPV genotypes and to be sensitive enough to amplify as few as 62.5 copies of a plasmid.

Thus, the present primers may be useful in the detection of HPV infections, the identification of infective HPV genotypes, the epidemiological evaluation of HPV, the effectiveness and toxicity of developed HPV vaccines, and the like.

In another aspect, the present invention provides a method of detecting the HPV genome, which is based on performing a polymerase chain reaction (PCR) for DNA contained in a biological sample using one or more primer pairs selected from among pairs of primers capable of complementarily binding to the HPV genome and having the nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7

and 8.

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The term "biological sample", as used herein, includes, but is not limited to, samples, such as tissues, cells, whole blood, sera, plasma, saliva, sputa, cerebrospinal fluid, urine, or the like, of individuals infected with HPV or suspected of being infected with HPV, or individuals vaccinated with a HPV vaccine.

A method for identifying the presence and genotype of HPV is particularly not limited as long as it employs the aforementioned primers. Examples of such methods include direct identification of HPV DNA using a primer of a specific strand as a probe, Southern blotting, dot blotting, and filter in situ hybridization (FISH). Alternative methods include a method based on amplifying HPV DNA using a pair of primers, genotype-specific polymerase chain reaction (PCR), and general-primer PCR. PCR is most preferred.

The term "polymerase chain reaction (PCR)", as used herein, is a representative nucleic acid amplification technique (NAT), which enzymatically amplifies a specific DNA region of interest in vitro. The PCR method, which was developed in 1985 by Mullis et al., can amplify any segment of a DNA molecule if its boundary sequences are known. PCR basically consists of three major steps: denaturation, annealing and extension. A specific DNA sequence is amplified while these three steps are repeated. In the

first step (denaturation) of PCR, a double-stranded template DNA is denatured into two single strands. In the second step (annealing), primers anneal with the two kinds of single-stranded DNA, in which a sequence desired to be amplified is interposed between the primer binding regions. In the third step (extension), a heat-resistant DNA polymerase extends the primers and synthesizes the complementary strand of the target sequence. This cycle is repeated 25 to 30 times.

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Primers are the most important factor determining the reliability of PCR results. Some primer sequences can give rise to non-specific amplification, leading to false In this regard, the present invention provides results. reliable primer pairs. The performance of PCR with the primer pairs of the present invention enables accurate detection of HPV genotypes and sensitive quantitative analysis of very small amounts. Also, when PCR is carried out with the primer pairs of the present invention, consistent results are obtained upon repeated PCR performance. That is, since the primer pairs of the present invention are highly valid and reliable, the results obtained with the present primer pairs are highly reliable.

In a preferred aspect, the present invention provides a method of detecting the HPV 11 L1 gene using a pair of primers having the nucleotide sequences of SEQ ID Nos. 1 and 2.

In another preferred aspect, the present invention provides a method of detecting the HPV 16 L1 gene using a pair of primers having the nucleotide sequences of SEQ ID Nos. 3 and 4.

In a further preferred aspect, the present invention provides a method of detecting the HPV 18 L1 gene using a pair of primers having the nucleotide sequences of SEQ ID Nos. 5 and 6.

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In yet another preferred aspect, the present invention provides a method of detecting the HPV 31 L1 gene using a pair of primers having the nucleotide sequences of SEQ ID Nos. 7 and 8.

A PCR for amplifying an HPV gene, in detail the L1 gene, using the primers of the present invention may be carried out through an ordinary PCR method. Also, conditions including time, temperature and cycle number, under which denaturation, annealing and extension reactions are allowed to occur, may vary. In the present invention, PCR conditions included 35 cycles of denaturation at 94°C for 1 min, annealing at 51°C for 1 min, and extension at 72°C for 1 min or 1 min 30 sec.

In a further aspect, the present invention provides a kit for detecting the HPV genome, comprising one or more primer pairs selected from among pairs of primers capable of complementarily binding to the HPV genome and having the nucleotide sequences represented by SEQ ID Nos. 1 and 2,

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SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.

In addition to the primer pairs, the detection kit of the present invention is composed of one or more different compositions, solutions or instruments, which are suitable for analysis methods. Preferably, the kit of the present invention includes the following constituents: a container containing detection primers; amplification reaction tubes or other suitable containers; reaction buffer (pH and magnesium concentration of which may vary); dNTPs; an enzyme such as Taq-polymerase; RNase; and sterile water. More preferably, the kit may further include a plasmid carrying an HPV gene as a positive control in order to realize quantitative analysis. Such a plasmid may be one or more selected from among pGEM-HPV11 L1, pGEM-HPV16 L1, pGEM-HPV18 L1, and pGEM-HPV31 L1, which will be described in the following examples.

A better understanding of the present invention may be obtained through the following examples which are set forth to illustrate, but are not to be construed as the limit of the present invention.

EXAMPLE 1: Construction of recombinant HPV L1 plasmids (standard DNA)

PCR primers specific to low risk HPV 11 and high risk

HPV 16, 18 and 31 were designed based on major protein (HPV L1) sequences of the different genotypes of HPV, which are deposited in GenBank. In order to obtain HPV genotypes commonly found in Korean, tissues of Korean cervical cancer patients were obtained from clinical hospitals, and genomic DNA as an HPV genome source was extracted from the tissues. Biological tissue samples were paraffin sections or biopsy samples prepared for pathological examination. PCR was carried out using the extracted genomic DNA with primers having the nucleotide sequences of SEQ ID Nos. 9 to 16, which are listed in Table 1, below. As a result, PCR products of about 1.6 kb were obtained.

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TABLE 1
Primer sequences against the HPV L1 gene for the preparation of national (Korean) standard DNA

HPV genotype	PCR fragment length		PCR primer sequence
HPV 16	1596 bp	Sense	5'-GCCCCCAAGCTTGCCGCCACCATGCAGGTGACTTTTATTTA
111 4 10	1330 25	Anti-	5'-ATCGGGCTCGAGCAGCTTACGTTTTTGCGTTTAGC-3' (SEQ ID
		sense	No. 10)
		Sense	5'-GCCCCCAAGCTTGCCGCCACCATGTGCCTGTATACACGG-3' (SEQ
HPV 18	1707 bp	Selise	ID No. 11)
HPV 16	1707 bp	Anti-	5'-ATCGGGGAATTCCTTCCTGGCACGTACACGCACACG-3' (SEQ ID
		sense	No. 12)
		Sense	5'-GCCCCAAGCTTGCCGCCACCATGTCTCTGTGGCGGCCTAGC-3'
HPV 31	1515 bp	pelise	(SEQ ID No. 13)
ULA 21	1313 bp	Anti-	5'-ATCGGGGAATTCCTTTTTAGTTTTTTTACGTTTTGCTGGTGTAGTGG-
		sense	3' (SEQ ID No. 14)
		Sense	5'-GCCCCCAAGCTTGCCGCCACCATGTGGCGGCCTAGCGACAGC-3'
HPV 11	1506 bp	Delise	(SEQ ID No. 15)
HEV II	1000 pp	Anti-	5'-ATCGGGGAATTCCTTTTTGGTTTTGGTACGTTTTCGTTTGGG-3'
		sense	(SEQ ID No. 16)

PCR was carried out under the following conditions. PCR was carried out using the DNA samples extracted from tissues from patients as templates with 2.5 mM dNTP, reaction buffer, primer pairs (20 pmol) listed in Table 1, According to the optimal annealing and SuperTag Plus. temperature of primers, a cycle of denaturation at 94°C for 1 min, annealing at Ta for 1 min, and extension at 72°C for 1 min 30 sec was repeated thirty five times, followed by final extension at 72°C for 10 min. Each PCR product was (Promega, vector USA) and pGEM-T-Easy Plasmid DNA was then transformed into $E.\ coli$ DH5 $\alpha.$ isolated and digested with EcoRI to determine whether the PCR product was successfully inserted (Fig. 1).

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The nucleotide sequences of the PCR products were determined and compared with previously known nucleotide sequences coding for the L1 protein of HPV genotypes. HPV 16 was compared with AF402678, HPV 31 with J04353, HPV 11 with NC_001525, and HPV 18 with NC_001357. As a result, the nucleotide sequences coding for the L1 protein of the four HPV genotypes, which were identified according to the procedure described above, were found to be highly similar to the conventionally known nucleotide sequences coding for L1 proteins of the HPV genotypes.

EXAMPLE 2: Large preparation and quantification of the recombinant HPV L1 plasmids

E. coli cells transformed with the recombinant HPV L1 plasmids prepared in Example 1 were inoculated in 10 ml of LB medium supplemented with ampicillin, and grown in a shaking incubator at 37°C overnight. Plasmid DNA was then isolated using an alkaline lysis method, and precisely quantified using a spectrophotometer. The plasmid copy number was calculated according to Equation 1, below.

[Equation 1]

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Copy number of 1 kb fragment = (1000 bp \times 660 g/mole) 10 / (6.023 \times 10²³ molecules) = 1 \times 10⁻¹⁸ g (1 fg)

Equation 1 means the following. One copy of a 1 kb (1000 bp) plasmid weighs 1×10^{-18} g (1 fg), and one gram of 1 kb plasmid DNA contains 10^{18} copies of the plasmid.

Using Equation 1, the number of copies of each of different HPV genotype plasmids was calculated, and a plasmid solution having 2000 copies was serially diluted two-fold, thereby yielding $10-\mu\ell$ solutions containing 2000, 1000, 500, 250, 125 and 62.5 copies of the plasmids.

EXAMPLE 3: Evaluation of the sensitivity of specific primers using the recombinant HPV L1 plasmids

Using the 10 μ l plasmid solutions containing 2000,

1000, 500, 250, 125 and 62.5 copies, prepared in Example 2, PCR was carried out. As a result, PCR primers displayed sensitivity in a manner dependent on the number of plasmid copies (indicating that the 10 μ l DNA solutions respectively contained 2000, 1000, 500, 250, 125 and 62.5 copies of the plasmids). Referring to Fig. 6, PCR primers represented by SEQ ID Nos. 1 to 8 (Table 2) were determined.

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TABLE 2
Primers for detecting the HPV L1 gene

Genotype	Sense primer	Anti-sense primer
HPV 11	TTAGGCGTTGGTGTTAGTGG (SEQ ID No. 1)	AAAATTCATAGCACCAAAGC (SEQ ID No. 2)
HPV 16	TTAGGTGTGGGCATTAGTGG (SEQ ID No. 3)	AAAGTCCATAGCACCAAAGC (SEQ ID No. 4)
HPV 18	TTAGGTGTTGGCCTTAGTGG (SEQ ID No. 5)	AAAGTCCATGGCACCATATC (SEQ ID No. 6)
HPV 31	TTAGGTGTAGGTATTAGTGG (SEQ ID No. 7)	AAAATCCATAGCTCCAAAGC (SEQ ID No. 8)

PCR was carried out as follows. 5 μ l of 2.5 mM dNTP was mixed with 5 μ l of 10 × buffer, primers (20 pmol) of SEQ ID Nos. 1 to 8, 0.5 μ l of Taq polymerase, and distilled water to give a final volume of 40 μ l. The mixture was supplemented with 10 μ l of each template, thereby yielding a PCR mixture. PCR conditions included 35 cycles of denaturation at 94 °C for 1 min, annealing at 51 °C for 1 min, and extension at 72 °C for 1 min, followed by final extension at 72 °C for 10 min. PCR products were then separated on a 1.5% agarose gel for 40 min, and stained with ethidium bromide (EtBr). Band intensity was measured

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using the software, Quantity One (Bio-Rad). regression function was derived in order to determine the relationship between band intensity and the number of plasmid copies, and a relative coefficient R was calculated to determine whether it was greater than 0.9. As a result of the sensitivity test for the PCR amplification method, the band intensity, as shown in Fig. 7, decreased in all of the four HPV genotypes in a manner dependent on the number of plasmid copies, and this PCR method was found to have a sensitivity detecting as few as 62.5 copies of the HPV L1 plasmids. When DNA was run on an agarose gel to determine the relationship between band intensity and the number of plasmid copies, the relative coefficient, as shown in Fig. 7, was greater than 0.9. These results indicate that a plasmid copy number test using the method of the present invention provides reliable results.

EXAMPLE 4: Evaluation of the specificity of primers using the recombinant HPV L1 plasmids

In order to determine whether the primers used in Example 3 specifically amplify each HPV genotype, the primer sets to the four different HPV genotypes were evaluated for whether they differentially amplify L1 templates of different HPV genotypes. PCR was carried out under the same conditions as in Example 3 except that the

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templates were used in a concentration of 1000 copies. was performed with each primer set using each of the four different HPV genotypes as a template. As a result of the PCR with each primer set using 1000 copies of each HPV genotype as a template, all primer sets to HPV 11, 16, 18 and 31 were found to specifically amplify only their corresponding templates (Fig. 8). The results, specifically that the primers of the present invention precisely detect only their corresponding HPV genotypes under optimized PCR conditions, indicate that the different primer sets enable clinical samples and differential genotyping of detection of high risk HPV types 11, 16 and 18, infections of which are most likely to progress to cervical cancer. Thus, the present primers may become a very useful means of clinical diagnosis.

EXAMPLE 5: Evaluation of the heat stability and long-term preservation of the recombinant HPV L1 plasmids

To evaluate the heat stability and long-term preservation of primers, first, 30 μ l of 1000 copies of each HPV L1 plasmid was aliquotted into fifteen DNase/RNase-free vials. The containers were stored at 4°C, 22°C and 37°C for a heat stability test, and at -80°C for long-term storage. Every three weeks, one vial at each storage temperature was subjected to a sensitivity test, which was carried out

according to the same procedure as in Example 3. The PCR with plasmids stored at 22° C and 37° C for three weeks showed negative results, indicating that standard DNA has a very low stability when stored at 22° C and 37° C (Fig. 9). In contrast, standard DNA stored at 4° C and -80° C for 15 weeks still provided highly sensitive PCR results, indicating that the standard DNA of the present invention is stable when stored at -80° C for a long period of time (Figs. 10 and 11).

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10 EXAMPLE 6: Evaluation of the applicability of primers using the recombinant HPV L1 plasmids

The procedure with the HPV L1 templates and primers in the present invention was evaluated to determine whether it could detect the HPV genome in human clinical samples, as follows. Genomic DNA was extracted from human rhabdomyosarcoma (RD), HeLa and SLK cells using genomic DNA extraction kit (Qiagen), the determined using concentration thereof was spectrophotometer. Each DNA solution was diluted to concentrations of 10 ng and 100 ng. 100 μ l of each dilution was aliquotted and stored at $-20\,\mathrm{C}$. Then, PCR was carried out with 100 ng and 1 $\mu\mathrm{g}$ of genomic DNA background using the same templates as in the sensitivity test of Example 3. PCR products were analyzed according to the same method used

for the sensitivity test. In the PCR with the genomic DNA background, the HPV templates, as shown in Fig. 12, could be detected even with as few as 62.5 copies, as in the absence of human genomic DNA. The PCR with genomic DNA background from HeLa cells, which contain the HPV 18 genome, exhibited positive results in all lanes.

Industrial Applicability

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As described hereinbefore, the primer pairs specific to the L1 gene of HPV 11, 16, 18 and 31 according to the present invention may be useful in the detection of HPV infections, the identification of infected HPV genotypes, evaluation of the effectiveness and toxicity of developed HPV vaccines, and the like.

Claims

1. A primer pair selected from among pairs of primers capable of complementarily binding to human papillomavirus (HPV) genome and having nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.

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- 2. A kit for detecting human papillomavirus (HPV) genome, comprising one or more primer pairs selected from among pairs of primers capable of complementarily binding to the HPV genome and having nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.
- 3. A method of detecting human papillomavirus (HPV) genome, comprising performing a polymerase chain reaction for genomic DNA contained in a biological sample using one or more primer pairs selected from among pairs of primers capable of complementarily binding to the HPV genome and having nucleotide sequences represented by SEQ ID Nos. 1 and 2, SEQ ID Nos. 3 and 4, SEQ ID Nos. 5 and 6, and SEQ ID Nos. 7 and 8.
- 4. The method as set forth in claim 3, wherein an HPV 11 L1 gene is detected using the primer pair having the

nucleotide sequences represented by SEQ ID Nos. 1 and 2.

5. The method as set forth in claim 3, wherein an HPV 16 L1 gene is detected using the primer pair having the nucleotide sequences represented by SEQ ID Nos. 3 and 4.

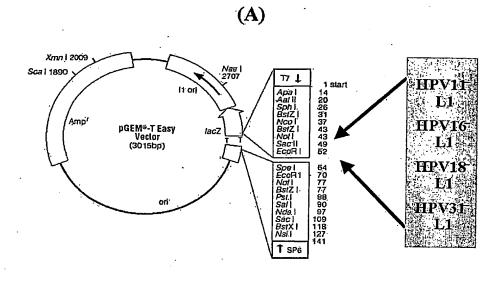
- 6. The method as set forth in claim 3, wherein an HPV
 18 L1 gene is detected using the primer pair having the
 nucleotide sequences represented by SEQ ID Nos. 5 and 6.
 - 7. The method as set forth in claim 3, wherein an HPV 31 L1 gene is detected using the primer pair having the nucleotide sequences represented by SEQ ID Nos. 7 and 8.

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8. The kit for detecting the HPV genome as set forth in claim 2, further comprising one or more plasmids selected from among pGEM-HPV11 L1, pGEM-HPV16 L1, pGEM-HPV18 L1 and pGEM-HPV31 L1 as a positive control in order to realize quantitative analysis.

Fig. 1



(B)

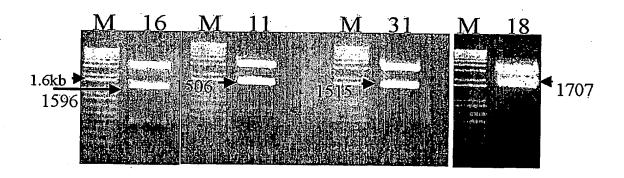


Fig. 2

	1	10	20	30	40	50	60	70	B0	50	100	110	120	130
EF402678 EPV16 Consensus									TTCRGNTSTC TTCRGNTGTC TTCRGRTGTC					
	131 1	40	150	150	170	180	190	200	210	220	· 230	240	250	260
EF402578 EPV16 Consensus									NETTGERGTTO RETTGERGTTO NETTGERGTTO					
	ļ	70 	280	230	300	310	320	330	340	350	350	370	380	390
RF402678 RPV16 Consensus									CCTGRCACCTO CCTGRCACCTO CCTGRCACCTO					
	I	00	410	420	430	440	450	450	470	480	430	500	510	520
RF402678 HPV15 Consensus									CREADANTECT CREADANTECT CREADANTECT					
	1	30	540	550	580	570	580	530	600	61.0	620	530	640	650
RF402678 HPV16 Consensus									TECECATETA TECECATETA TECECATETA					
	1	50	670	580	630	700	710	720	730	740	750	760	770	780
RF402678 KPV16 Consensus									INCRRARACTER INCRRARACTER INCRARACTER					
	781 7	30	800	810 ·	B20	B30	840	850	850	870	980	890	900	910
RF40267B HPV16 Consensus	111 1111111111111	յն I Ե I Ա Ա	шшип	6CG ILLIEG TT	411111111	T FOR CHARGE	anconarci	TTCTTACCACA	TTTATTTAAT TATTTATTTA	DECEPTEETC	CYCTTEETEC	COUNTERPORCE	COCCUPTANT	DOUTTO
	911 9	20	930	940	950	960	970	500	990	1000	1010	1020	1030	1040
RF402679 HPV16 Consensus	HOUSELL CT	EGG I CTHC	1 SCRHHTII	RUSCE RETTER	RETTRITTE	CTRCACCTAG	TESTICION	GGTTDFFTF	GATGCCCARA GATGCCCARA	TOTTCOOTOC	MCCTTOTTCC	TYDEDOCCOC	******	CONTRO
•	1041 105	50	1060	1070	10B0	1090	1100	1110	1120	1130	1140	1150	1160	1170
RF402678 HPV16 Consensus	I GULLIII I I	ri i lubbub i	нисавили	11161112216	TELLERIE	TREMETERS	remaren	TEATTATETE	ETGECATATE ETGECATATE ETGECATATE	TRETTEREN	OFTOPOTOTO	COORTOCIO	CYYYOUCCOC	TOCCTO
	1171 118	30	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290 .	1300
RF402578 HPV16 Consensus	CHILITIES			554 I I I I I I I I I I I I I I I I I I	LHOULIGIBL	100001110REET	TRUE HELDING	NISTIBIER	OTRENTACOT OTRENTACOT ATRENTACOT	TETRTERRIT	CERCIPITY	CENTERCYTE	COTTTTCCTP	TDCDGC
	1301 131	.0 :	1320	1330	1340	1350	13E0	1370	1380	1390	1400	1410	1420	1430
RF402678 HPV16 Consensus		anustru	at incomen	BCTTOTES:	TISTERCRI	CCCREECONY	TECTTETER	AGASTATATE	CETCERGERE CETCERGERE CETCERGERE	TODOCCOR.	TECEFTTOON	ODOTOPOPYT	TTTTCCCCCCC	DOCTTO
	1431 144		1450		1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
RF402678 ICPV16 Consensus									CCRRRCCOARI CCRRRCCOARI CCRRRCCARA					
	1561 157	0 1	L580	1594593				•						•
RF402678 HPV16 Concensus	TETREARCT TETREARCT TETREARCT	GCTARACO	CENTRARACE CENTRARACE CONTRARACE	TARGETG										

Fig. 3

	1	10	20	30	. 40	50	60	70	80	90	100	110	120	130
J04353 ISPV31_11 Consensus	HILL		GECT REFER	ari Britir	metnasse	PTCTPPPPPP	TETAGORET	TGTRAGENEGG TGTRAGENEGG TGTRAGENEGG	atreca mitet	BBCBCCccccc	ορεατοτοτές	TERRETORY	THE PROPERTY OF THE PARTY OF TH	TEETTE
	131	140	150	160	170	180	190	200	210	220	230	240	250	260
JO4353 HPV31_L1 Consensus	aui		Barrer CC	IIII CETARAT	a ar sia talla da	TOROROSHTO	TISTOCIS	RAGE 10 TCDES RAGE TE TCRES RAGE TG TCAGS	ATIACONIAT	BELLIBERT	SCETTEGITTE	CERCOTECOS	MCDOGTTYCE	WITTE
	261	270	280	290	300	310	320	330	340	350	360	370	380	330
J04353 HPV31_L1 Consensus	1 1 1 1 1 1	11.001.661471	H112811 LL 112	OUGLE CIRCLE	Criuci i usi	eccietata	TITOGRAFIA	INGGTCGCGGG INGGTCGCGGG INGGTCGCGGG	TRECEDITAG	CTETOCCTOT	roct cet cott	TOTTOTTOOL	11000171601	COCOCY
•	391 	400	410	420	430	440 .	450	450	470	480	490	500	510	520
J04353 HPV31_L1 Consensus	ыню	ŒIET(BITAL	018163653	DESTRUCTION OF	TETENTRATA	CCCONTENT	ITERRITARI	TTATRABERDA TTATRABERDA TTATRABERDA	COCONCICIO	TITOPTT CCT	reconnectors	TOTTCCCC	POTTCCCCTO	DOCCTO
	521	530	540	550	560	570	580	590	600	610	- 620	630	640	650
J04353 RPV31_L1 Consensus	SILE	TGTHSTHRE	BELLEE LIBIT II	THE PERSON OF TH	3116166166	artnanattae	101111111111	TTATREARCA TTATREARCA TTATREARCA	rceccerate	TTTCO TOCOCI	CTTTTCCCCCTT	THEOTYPE	PETECHTTOCO	ocococ
*	651	660	670	680	690	700	710	720	730	740	750	760	770	780
J04353 HPY31_L1 Consensus	111111111111111111111111111111111111111	GIRCHESTIC	CITTGGRCAT	HELANITE	MILLERMAN	TATCCCCCATTE	TETTBORRY	GETTECTENSI GETTECTENSI GETTECTERSI	CATATGGCE	TOPPTTOTT	TTYTOTTTO	THE OCCUPANTE	ROTEYTTETE	OCECOY.
	781	790	800	810	820	830	840	650	860	B70	BBO	B90	900	910
J04353 HPV31_L1 Consensus	11111	1 1 1 1 1 1 1 1 1 1 1 1 1 1 C	1666CHC661	GGIGRAICS	FICCUTRUEG	BETTATATAT	NUNCTICE TO	GGTTERRENGI GGTTERRENGI GGTTERRENGI	TRETTYRRE	TRACASTACAT	RETTTECYRE	OCC TREFER	TECRTESTER	202117
	911	920	930	940	950	680	970	980	990	1000	1010	1020	1030	1040
J04353 HPV31_L1 Consensus	MIGGE	CHART TITT	ANTARACCAT	DITEGRICO	MEGTECTER	GEGREACACAAA	MIGGIAITI	GTTGGGGCRA GTTGGGGCRA GTTGGGGCRA	CRETTATITE	TYPETETER	ACUTOCORO	CCYACTOCCO	MIDIEICTET	TTCTCC
	1041	1050	1050	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
J04353 HPV31_L1 Consensus	TGCR	ITTGCARALIN	STGRYNCTRO	ATTTANARGI	RETRATTAL	MAGRETHITT	RAGRESTRO	TGRGGANTTT TGRGGANTTT TGRGGARTTT	CERTITION OF THE	TREATTECHT.	TYNTECHNOR	TARCATTATE	TERREBERRE	OTHER
	1171	1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300
J04353 HPV31_L1 Consensus	TATAL	TENCHISTATI	GNATECTECI	ATTIT GGILLIC	RTTGGARTT	TTEGRTTGDCC	REDCETEE	TCAGGTTCTTT TCAGGTTCTTT TCAGGTTCTTT	GEREGATACI	TATAGGTTTE	TORCETCACA	SGCCOTTACA	TOTCARBARO	TECCC
	1301	1310	1320	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430
JO4353 HPY31_L1 Consensus	CCCRF	MRGECERNGI	INGATECAT	TTRANSATTA	ITGTATTTTGI	CONCUTABLE	TAAANGAAR	AGTITICTECE AGTITICTECE AGTITICTECE	GATTTREATE	ASTITCCACI	GGGTEGERAN	TTTTTTTRC	AGGCAGGATAI	REGEE
	1431	1440	1450	1460	1470	1480	1490	1500	151812					
J04353 HPV31_L1 Consensus	RESTE	CTONATTIN	MGCAGGTAR	BCGTRGTGCI	CCCTCNGCA1	CTRCCRCTRC	ACCOCCOOR	NCG TARANARA RCG TARANARA RCG TARANARA	CTRARARS					

Fig. 4

	<u>1</u>	10	20	30	40	50	60	70	80	90	100	110 .	120	130
HC_001525 HPV11_L1 Consensus	ATET ATET	EGCEGECTRE ESCGECCTRE GSCGGCCTRE	CENCRECAC CENCRECAC	ACTATATOTES ACTATATOTES ACTATATOTES	ETCETCECE ETCETCECE ETCETCECE	ACCC TETATO ACCC TETATO ACCC TETATO	CRAGGTTGT CRAGGTTGT CRAGGTTGT	TECCNICENTE TECCNICENTE TECCNICENTE	CETATETTAN CETATETTAN CETATETTAN	CGENEENRE CGENEENRE	OTATTTTBTC OTATTTTATC	ATGCCOGCOG ATGCCOGCOG	TETRENETE TETRENETO TETRENETO	TTECTE TTECTE
	131	140	150	150	170	180	190	500	21.0	220	230	240	250	260
HC_001525 HPV11_L1 Consensus									AGTETTTANGE BOTETTTANGE BOTETTTANGE					
	261	270	280	290	300	310	320	330	340	350	360	370	380	330
NC_001526 HPV11_L1 Consensus	GTTT	GACCECACTA							STEGIETTA STEGIETTA STEGIETTA					
	391 	400 '	410	420	430	440	450	460	470	480	490	500	510	520
HPV11_11 Consensus									TGGTGGGCTGT TGGTGGGCTGT TGGTGGGCTGT					
	521	530	540	530	560	570	5B0	530	600	£10	650	630	640	650
KC_001525 KPV11_L1 Consensus									TGNTACAGGCT TGNTACAGGCT TGNTACAGGCT					
	651	650	670	680	630	700	710	720	730	740	750	760	770	780
HC_001525 HPV11_L1 Consensus									NSGTTGTTTTT NSGTTGTTTTT					
	781	790	800	810	820	830	840	850	860	870	880	690	900	910
KC_001525 KPV11_L1 Consensus									TAGTATTAT TAGTATTAT TAGTATTAT					
	911	920	930	940	950	350	970	980	930	1000	1010	1020	1030	1040
RC_001525 RPV11_L1 Consensus									TACTGTGGTNG TACTGTGGTNG TACTGTGGTNG					
	1041		1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
NC_001525 HPV11_L1 Consensus									TTCANTIGIG TTCANTIGIG TTCANTIGIG					
	1171		1190	1200	1210	1220	1230	1240	1250	1260	1270	12B0	1290	1300
									ATOTETRICAS ATOTETRICAS ATOTETRICAS					
	1301	1310	1320	1330	1340	1350	1350	1370	1380	1390	1400	1410	1420	1430
									CCCCTTGGACO CCCCTTGGACO CCCCTTGGACO					
	1431		1450	1460	1470	1480	1490	1501503						
NC_001525 IPV11_L1 Consensus	TREAG	STATBRAGCG	CCCAGCTGT	STETMRGECET STETMRGECET STETMRGECET	CTRCRECCE	CORREGERRE	REG TRECAR	DRECKRRARG						

Fig. 5

	1	10	20	30	40	50	- F0	70	<u> </u>	90	100	110	120	130 1
NC_001357 HPV18L1-0	BLELECC	TOTHTREE	COGNICCION COGGICCION	TATTACATTI TATTACATTI	CCRICTACE	RECTETOTAT RECTETETAT	EECCCNTT 61	HICHECORU	BECCCCTECCT	CTRCHCRSTI CTRCHCRGTI	ITATTESTAT	RCHTGGTNCH NOTTGGTNCH	CATTATTATTI COTTATTATTI	TETESCC TETESCC
KPV1RL1-L Consensus	ATETEC	TETOTOCO	CEGGT CCT CA	TATTREATT	CONTETAC.	ACCTETATO	GECCCONTTG!	ATCOCCONC	.60000016001	TETRERERGTI	THITTEGTHT	NCATEGTACA	COTTOTTALT	TET GECC
	131	140	150	160	170	100	190	200	210	220	230	240	250	260
HC_001357 HPV18L1-U	arima	патттат	TECTONERAR	CGTRANCGT	TTECETATI	TTTTTECHEN	TESE ET TETT	6CGECCTRS	IGNORATION	TATAICTTC	RECTECTIC	TETESCHALL	GITGTRARITO GITGTRARITO	CONTOR
HPV18L1-L													GTTGTAAATAC	
Conscisus	261	270	280	298	200	310	320	330	340	350	350	370	380	290
NC_001357	1												REGITTETEC	
HPV181.1-U	imigie	ACT COCAC	RAGCATATTT	micmeet	GENGETETO	GRITATTRAC	TETTEETRAT	CONTRITTI	RESUTTECTED	NGGT GGT GG	ANTONGONG	GRIBITOCTA	REGOTTTCTEC	TRECEN
Consensus		mete.cne	DOSCRIBITY	TATCATECT	GCREETC IN	SATTATTARE	TETTGETADI	COTRITTI	REGETTECTE	TREGTEGTED!	RATARGEAG	COTATICCIA	NGSTTTC TGCT	TACCAN
	391	400	410	420	430	440	450	450	470	480	430	500	510	520
HPV18L1-U	TATAGAS	TATTIAGE TATTIAGE	STECRETTRE STECRETTRE	CTERCECRAI CTERCECRAI	TRARTTICS TRUBITICS	TTTRECTGAT TTTRECTGAT	RETRETATTI RETRETATTI	RTRATCCTG NTOATCTTG	MACACARCE! RANCACARCE!	TTRETETER	CCTGTGCTG CCTGTGCTG	GRGT GGRRAT GRGT GGRRAT	TEGECETGETC	RECETT
RPV1BL1-L Consensus	TOTAGRE	TOTTTOGG	GTECRETTRE	CTGACCCRAF	TREATTEG	TTTRCCTGAT	ACTRETATTI	TTARTC.TG	MACAENNEG1	TERETETEC	CCTGTGCTG	GRGTGGARAT	TESCCETGETE	RECETT
	521	530	540	550	560	570	580	590	600	610	620	630	640	650
NC_001357	TREGTET	TGGCCTTR	CTEGGCATCC	OTTTTATEA	ILDATTRICAT	GRENCTGRRR	GTTCCCNTGC	CGCCACGTC	MATGTITCT	AGGACGTTIN	EGRCERT ST	GTCTBTAGRT	TATBRECASAL	RCRGTT
HPV18L1-U HPV18L1-L									TRATGTTTCTE TRATGTTTCTE					
Consonsus		660	670	ERO BITTERINGO	620	700	710	720	730	740	, 750	750	770	780
HC_001357	1												TTTTGGRHGR	1
HPV18L1-U	4101011	11000010	I GLLLL I GL I	H 1 I BURGISH	ur i ener i u	underlier i er	i io i mai i ci	LBILLIII	t ruthonarbt	111000000	i i i manne i i	COMPANIAL NO	11110000000	1001011
Consensus														
	781	790	800	810	B20	830	840	850	850	870	880	890	900	910
NC_001357	BYSGTBS	ATACTEGA	TATEGT 6CCA	TGENETITRE	TROSTTECA	HENTRETRRA	TETGREGTRE	CATTEGATA	TTTGTCRETCT	ATTTGTARA	RTCCTGATT	ATTTRCARRI	STCTGCRGATO	CTTATE
IPV1BL1-L Consensus														
	911	920	930	940	950	360	970	980	590	1000	1010	1020	1030	1040
NC_001357	GGENTTO	CHIGITIT	TTTGCTTACG	GCGTGRGCRI	CTTTTTGCT	RGECOTTTTT	GGRATRGBGU	RESTRETATI	GEGTERCRETE	TECCTCAST	CTTRTATAT	TARREGERER	EGTATECCT GE	CTTCRCC
HPV18L1-U HPV18L1-L														
Соласпаца														
	1041 1					1030			1120	1130	1140			
NC_001357	1	1050	1060	1070	1080		1100	1110				1150	1150	1170
11PV181.1-U	TEECHE							_=;			EGTCATRAC		1160 GCTGGCRTART	
NPV18L1-U KPV1BL1-L Concencus	TEECHE							_=;			EGTCHTRAC			
KPV1BL1-L								_=;			1270			
KPV18L1-L Concencus	1171 1	1616TGTA	1190	1200	1210	LCTCTENCTC	CCAGTTGTTT	1240	TRYTEGITACE	TANGGEREN 1260	1270	RATGGTGTTT	GCTGGCRTRAT	1300
HPV181.1-L Consensus HC_001357 HPV181.1-U HPV181.1-L	1171 1	1616TGTA	1190	1200	1210	LCTCTENCTC	1230 RERERGIETE	1240 CTGTACCTG	1250 GGCARTATGAT	1260 GCTACCRAN	1270 TTRRGCRGT	1280 HTAGCAGACA	1290 TGTTGRGGAN	1300
HPV181.1-L Concencus NC_001357 HPV181.1-U	1171 1 	LISO ECTETGETR	1190 SHTRECRETE	1200	1210 ITTBACART	1220 HIGTGEVICT	1230 RCRCRGTCTC CRSTCTC	1240 CTGTACCTG	1250 GGCARTATGAT GGCARTATGAT GGCARTATGAT GGCARTATGAT	1260 GCTACCRARI GCTACCRARI GCTACCRARI	1270 TTANGCRET TTANGCRET	1280 1280 NTAGCAGACA NTAGCAGACA NTAGCAGACA	1290 TGTTGREGAN TGTTGREGAN TGTTGREGAN	1300 TATGATT
HPV1811-L Consumcus NC_001357 HPV1811-U HPV1811-L Consensus	1171 1 111617F	LIBO ECTREGETR	1190 68TRCCRCTC	1200 CCRGTRCCRS	1210 ITTERCRAT	1220 RTGTGCTTCT	1230 REREAGIETE CRETETE CRETETE 1350	1240 CTGTACCTG CTGTACCTG CTGTACCTG	1250 GGCARTATGAT GGCARTATGAT GGCARTATGAT GGCARTATGAT 1320	1260 GCTACCAAN GCTACCAAN GCTACCAAN 1390	1270 TTARGCAGT TTARGCAGT TTARGCAGT	1280 ATAGERGACH ATAGERGACH ATAGERGACH 1410	1290 ITOTTGREGAN ITOTTGREGAN ITOTTGREGAN ITOTTGREGAN	1300 TRITGATT TRITGATT TRITGATT
HPV1811-L Concencus NC_001357 HPV1811-U HPV1811-L Consensus NC_001357 HPV1811-U	1171 1 1116177 1301 1	LISO ACTRIGGIA LISO	1190 SHTHECKETC 1320	1200 CCRGTRCCRS	1210 ITTERCRAT	1220 HTGTGETTET 1350 GTTGTGEET	1230 REGREGATETT CREATETT CREATETT 1350	1240 CTGTACCTG CTGTACCTG CTGTACCTG CTGTACCTG	1250 GGCRATATGAT GGCRATATGAT GGCRATATGAT GGCRATATGAT GGCRATATGAT GGCRATATGAT LGCRATATGAT	1260 TGCTACCAAN GCTACCAAN GCTACCAAN TGCTACCAAN TGCTACCAAN	1270 TTARGERGY TTARGERGY TTARGERGY TTARGERGY 1400	1280 RTAGGAGACA RTAGGACA RTAGCACA RTAGC	1290 ITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGREGARITGTTGTGREGARITGTTGTGREGARITGTTGTG	1300 TRIGATI TRIGATI TRIGATI TRIGATI TRIGATI TRIGATI
HPV1811-L Concencus HC_001357 HPV1811-U HPV18L1-L Consensus	1171 1 1116177 1301 1 1600677	LISO LISO LISO LISO LISO LISO LISO LISO	1190 SATRICCACTO 1320 SETTGTGTACCACTO	1200 CCRGTRCCAS 1330 TATTRCTTTE	1210 ITTTBBCBBT 1340 BCTGCBBT BCTGCBBT BCTGCBBT	1220 ATGTGETTET 1350 GTTATGTCET GTTATGTCET GTTATGTCET	1230 RESCRICTE CASTETT CASTETT 1350 RIBITICATAG	1240 CTGTACCTG CTGTACCTG CTGTACCTG TGTACCTG	1250 GGCARTATGAI GGCARTATGAI GGCARTATGAI 1320 CDGTATTTTAC	1260 TGCTRCCRAN GCTRCCRAN GCTRCCRAN 1390 INGGATTGGAL INGGATTGGAL	1270 TTANGCAST TTANGCAST TTANGCAST TTANGCAST TTANGCAST	1280 NTAGEREACH NTAGEREACH NTAGEREACH 1410 TECCCCCCCC	1290 ITOTTGREGAN ITOTTGREGAN ITOTTGREGAN ITOTTGREGAN	1300 TRIGATT TRIGATT TRIGATT TRIGATT TRIGATT TRIGAT
HPV18L1-L Consensus NC_001357 HPV18L1-U HPV18L1-L Consensus NC_001357 HPV18L1-U HPV18L1-U HPV18L1-L Consensus	1301 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	LIBO LIBO LIBO LIBO LIBO LIBO LIBO LIBO	1190 ERTRICACTO 1320 ERTTGTGTAC RETTGTGTAC RETTGTGTAC RETTGTGTAC	1200 CCRGTBCCRS 1330 TRITICETTIS TRITICETTIS TRITICETTIS 1450	1210 ITTRACRAT 1340 INCTECRIGNY INCTECRIGNT INCTECRIGNT 1470	1220 RIGIGETYET 1350 GITATGICET 611ATGICET 1480	1230 RERERBICTO CRATETT CRATETT 1350 DIBITORIES RIPHTCHIRS RIPHTCHIRS RIPHTCHIRS 1490	1240 CTGTACCTG CTGTACGATTG CTGTA	1250 GGCRATATGAT GGCRATATGAT GGCRATATGAT 1320 CDGTATTTTAC CDGTATTTTAC CAGTATTTAC	1260 GCTACCHAN GCTACCHAN GCTACCHAN 1390 GGGATTGGA GGGATTGGA 1520	1270 TTANGCAST TTANGCAST TTANGCAST 1400 ECTTGGTGT ECTTGGTGT	1280 HTHECHEACH HTHECHEACH HTHECHEACH 1410 HECCECCCC HCCCCCCCCCCCCCCCCCCCCCCCCCCC	1290 ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN 1420 ECGNETTRETRE ECGNETTRETRE	1300 INTEGRIT INTEGRIT INTEGRIT 1430 INTEGRIT IN
HPV181L Consensus NC_001357 HPV181.1-U HPV181.1-U Consensus NC_001357 HPV181.1-U Consensus	1171 1 1176176 1301 1 1- 16CRGTT 16CRGTT 1431 1 1- 16CRGTT	LIBO LIBO LIBO LIBO LIBO LIBO LIBO LIBO	1190 ERTRECERCTE 1320 RECTIGIGATE RECTIGIGATE RECTIGIGATE 1450 TOTRECRATET	1200 CCRGTBCCRC 1330 TRITRETTIF TRITRETTIF 1450 GTTGCTATYC	1210 ITTERCERT 1340 IRCTECTECTE IRCTECTECTE 1470 CCTUTCRER	1220 ATGRETTET 1350 GITATGRETTET 611ATGREET 1480 ASSATGETSC	1230 RECREGETETT CRATETT CRATETT CRATETT 1350 RITHTENTRE RITHTENTRE RITHTENTRE 1490 RECEGETER	1240 CTGTACCTG CTGTACCTG CTGTACCTG CTGTACTG 1370 CTGTACTGTATGATTAGATTAGATTAGATTAGATTAGAT	1250 GCCARTATORI GCCARTATORI GCCARTATORI GCCARTATORI 1320 CMGTOTTTRE CMGTOTTTRE CAGTATTTRE	1260 GETRECHRAN GETRECHRAN GETRECHRAN 1390 GREGATYCHRAN GREGATYCHRAN 1520 GETRRARGTY	1270 TTARGERST TTARGERST TTARGERST 1400 ECTITGGTGT ECTITGGTGT 1530 TGGGRRTGTG	1280 RTAGCREATE RTAGCR	1290 ITGTTGREGARI TGTTGREGARI TGTTGREGARI TGTTGREGARI 1420 ECCURCTRETRE ECCRETRETRE 2.018CTRETRE 3.050 ARRIBGTTTCT	1300 1816811 1816811 1430 1711061 1711061 1560
HPV18L1-L Consencus NC_001357 HPV18L1-U KPV18L1-L Consensus NC_001357 HPV18L1-U HPV18L1-L Consensus	1171 1 TITGITE 1301 1 TGCRGTT TGCRGTT TGCRGTT 1431 1 GGATACE	LISO CETTEGETH LISO CETTEGETH	1190 ERTRECERCT 1320 ERTTGTGTACE AGGTTGTGTACE AGGTTGTGTACE 1450 TGTACCATTCT	1200 CCRGTBCCRS 1330 TATTRETTIF THITATTITE 1450 OTTECTATTE OTTECTATTE	1210 ITTERCRAT 1340 ACTECRAT ACTECRAT 1470 CCTUTCRAR	1220 ATATACTECT 1350 ATATACTECT 611ATACTECT 1480 ASSATCTOCT	1230 RECRETETT CRETETT CRETETT 1350 RITHITICHTES RITHITICHTES 1450 RECEGETGRE	1240 CEGERACETGE CEGERACETGE CEGERACETGE CEGERACETGE CEGERACETGE 1370 INTEGRATISSE INTEGRATISSE 1500 ARTERISSERT	1250 GGCARTATORI GGCARTATORI 1380 LOGIGITITIA LOGIGIATITIA LOGIGIATITIA LOGIGIATITIA LOGIGIATITIA LOGIGIATITIA LOGIGIATITIA LOGIGIATICA LOGIGIA LOGIGIATICA LOGIGI	1260 GETACCHANI GETACHANI	1270 TTERRECRET TTERRECRET TTERRECRET TTERRECRET 1400 ECTTERFOR ECTTERFOR TESTS 1530 TTERRECRET TESTS TTERRECRET	1280 BTAGCREACH BTAGCR	1290 ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN 1420 ECGNETTRETRE ECGNETTRETRE	1300 IRTGATT
HPV1811-L Consencus NC_001357 HPV1811-U HPV1811-L Consensus NC_001357 HPV1811-U HPV1811-U HPV1811-U HPV1811-U HPV1811-U	1301 1 1	LISO CETTEGETH LISO CETTEGETH	1190 ERTRECERCT 1320 ERTTGTGTACE AGGTTGTGTACE AGGTTGTGTACE 1450 TGTACCATTCT	1200 CCRGTBCCRS 1330 TATTRETTIF THITATTITE 1450 OTTECTATTE OTTECTATTE	1210 ITTERCRAT 1340 ACTECRAT ACTECRAT 1470 CCTUTCRAR	1220 ATATACTECT 1350 ATATACTECT 611ATACTECT 1480 ASSATCTOCT	1230 RECRETETT CRETETT CRETETT 1350 RITHITICHTES RITHITICHTES 1450 RECEGETGRE	1240 CEGERACETGE CEGERACETGE CEGERACETGE CEGERACETGE CEGERACETGE 1370 INTEGRATISSE INTEGRATISSE 1500 ARTERISSERT	1250 GGCARTATORI GGCARTATORI 1380 LOGIGITITIA LOGIGIATITIA LOGIGIATITIA LOGIGIATITIA LOGIGIATITIA LOGIGIATITIA LOGIGIATITIA LOGIGIATICA LOGIGIA LOGIGIATICA LOGIGI	1260 GETACCHANI GETACHANI	1270 TTERRECRET TTERRECRET TTERRECRET TTERRECRET 1400 ECTTERFOR ECTTERFOR TESTS 1530 TTERRECRET TESTS TTERRECRET	1280 BTAGCREACH BTAGCR	1280 ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN 1487 LAVE CENTETRETAL CENTER CENTETRETAL CENTETRE	1300 IRTGATT
HPVIBIL-L Consences NC_001357 HPVIBIL-U HPVIBIL-U HPVIBIL-U HPVIBIL-U HPVIBIL-U HPVIBIL-U HPVIBIL-U Consensus	1301 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	LISO LISO LISO LISO LISTITUTO LISTIT	1190 ENTRECECTET 1190 ENTRECECTET 1320 ENTRETETETETETETETETETETETETETETETETETET	1200 CCRGTBCCRC 1330 TRITISCTITE TRITISCTITE 1450 TRITISCTRITE TRICTRITE TRICTRITE TRICTRITE TRICTRITE TRICTRITE TRICTRITE TRICTRITE	1210 17THRACRAT 1340 RCTGCHGAT RCTGCHGAT ACTGCTGCTGAT 1470 CCTGTCGGAT CCTGTCGGAT LGCTGTCGGAT	1220 ATGRICETTET 1350 GITATGRICET GITATGRICET 1480 L480 ASSATIACTOC ASSATIACT	1230 RERERBICTO CRATETT CRATETT 1350 RITHITENTER RITHITENTER 1450 RECEGETGER RECEGETGER RECEGETGER	1240 1240 CTGTACCTG CTGTACCTG CTGTACCTG 1370 INTERNATION INTERNATION INTERNATION ANTERNACION ANTERNACION ANTERNACION ANTERNACION INTERNACION INTERN	1250 GECRATATION GECRATATION GECRATATION GECRATATION 1330 CONTITUTE CONTITUT	1260 1260 1260 1260 1260 1260 1260 1390 1390 1390 1390 1500 1520 1520 1520 1520 1520 1520 1520 1520 1520	1270 TTARGERST TTARGERST TTARGERST 1400 ETTTGGTGT ETTTGGTGT 1530 TTGGARTGTG TGGARTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT	1280 BYRGERERER BYRGERERER BYRGERERER 1410 TECCCCCCC ICCCCCCCCC 1540 GRITTRANSG GRITTRANSG GRITTRANSG	1290 TIGTTGRGGAT TIGTTGRGAT TIGTGRGAT T	1300 IRTGATT FATGATT FATGATT FATGATT FATGATT FATGATT FATGATT FATTGGT F
HPVIBLI-L Concencius NL.001357 HPVIBLI-L HPVIBLI-L L Consensus NC.001357 HPVIBLI-L Consensus	1301 1 1	LISO LISO LISO LISO LISO LISO LISO LISO	1190 1320 1320 1320 1320 1320 1320 1320 132	1200 CCRGTBCCRC 1330 TRITICTITE TRITICTITE 1450 GTTGCTRTTE GTTGCTRTTT GTTGCTRTT GTTGCTT GTTGCTRTT GTTGCTT GTTGCTT GTTGCTT GTTGCTT GTTGCTT GTTGCT GTT	1210 1711BACARI 1340 ACTECHGAT ACTECHGAT 1470 CCTUTCARA CCTGTCARA CCTGTCARA CCTGTCARA CCTGTCARA CCTGTCARA CCTGTCARA CCTGTCARA	1220 1220 1350 1350 1350 1361 1350 1361 1360 1360 1360 1360 1360 1360 136	1230 RERERBICTO CRATETT CRATETT 1350 RITHTUTHER RITHTUTHER 1450 RECEGETGRIF	1240 LEGIACETG LEGIACETG LEGIACETG LEGIACETG LITTORNIAGE LITTORNIA	1250 GGCARTATGAT GGCARTATGAT GGCARTATGAT 1330 CONTATTATGAT 1310 CONTATTATGAT 1510 CONTATTAGAT CONTATTAGAT 1540 CONTAGAT 1640 CONTAGAT 1640	1260 1260 1360 1360 1370 1390 1390 1390 1320	1270 TTIARGERST TTARGERST TTARGERST 1400 ECTTEGIST 1530 TEGGRATTEG TEGGRATTEG TEGGRATTEG TEGGRATTEG TEGGRATTEG TEGGRATTEG	1280 HTMSCREAGHER HTMSCREAGHER HTMSCREAGHER 1410 TECCECCECE TECCECCECE 1540 GRITTHRANGG GRITTHRANGG GRITTHRANGG 1670 CTICTHRANGG CTICTHRANGG	1280 ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN 1420 ECHIETRETER ECHIETRETER ECHIETRETER ECHIETRETER ARABITTTTCT ARABITTTTCT 1680	1300 INTERNITY INTERNITY 1430 INTERNITY INTERNITY 1500 INTERNITY I
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NC.001357 HPVIRI.1-U FPVIRI.1-U Consensus NC.001357 FPVIRI.1-U Consensus	1300 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	LISO LISO LISO LISO LISO LISO LISO LISO	1190 1320 1320 1320 1320 1320 1320 1320 132	1200 CCRGTBCCRC 1330 TRITICTITE TRITICTITE 1450 GTTGCTRTTE GTTGCTRTTT GTTGCTRTT GTTGCTT GTTGCTRTT GTTGCTT GTTGCTT GTTGCTT GTTGCTT GTTGCTT GTTGCT GTT	1210 1711BACARI 1340 ACTECHGAT ACTECHGAT 1470 CCTUTCARA CCTGTCARA CCTGTCARA CCTGTCARA CCTGTCARA CCTGTCARA CCTGTCARA CCTGTCARA	1220 1220 1350 1350 1350 1361 1350 1361 1360 1360 1360 1360 1360 1360 136	1230 RERERBICTO CRATETT CRATETT 1350 RITHTUTHER RITHTUTHER 1450 RECEGETGRIF	1240 LEGIACETG LEGIACETG LEGIACETG LEGIACETG LITTORNIAGE LITTORNIA	1250 GGCARTATGAT GGCARTATGAT GGCARTATGAT 1330 CONTATTATGAT 1310 CONTATTATGAT 1510 CONTATTAGAT CONTATTAGAT 1540 CONTAGAT 1640 CONTAGAT 1640	1260 1260 1360 1360 1370 1390 1390 1390 1320	1270 TTIARGERST TTARGERST TTARGERST 1400 ECTTEGIST 1530 TEGGRATTEG TEGGRATTEG TEGGRATTEG TEGGRATTEG TEGGRATTEG TEGGRATTEG	1280 HTMSCREAGHER HTMSCREAGHER HTMSCREAGHER 1410 TECCECCECE TECCECCECE 1540 GRITTHRANGG GRITTHRANGG GRITTHRANGG 1670 CTICTHRANGG CTICTHRANGG	1280 ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN 1420 ECHIETRETER ECHIETRETER ECHIETRETER ECHIETRETER ARABITTTTCT ARABITTTTCT 1680	1300 INTERNITY INTERNITY 1430 INTERNITY INTERNITY 1500 INTERNITY I
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HPVIBIL-4. Consences NIC.001357 HPVIBIL-1 HPVIBIL-4. CONSENCES NIC.001357 HPVIBIL-4. HPVIBIL-4. CONSENCES NIC.001357 HPVIBIL-4. HPVIBIL	1301 1 1716TFF 1301 1 16CRGTT 16CRGTT 16CRGTT 16CRGTT 15TRGTT	LIBO CETTOTOGETH CETTOGETH CETTOGE	1190 1320 1320 1320 1320 1320 1320 1320 132	1200 CCRGTBCCRC 1330 TRITICTITE TRITICTITE 1450 GTTGCTRTTE GTTGCTRTTT GTTGCTRTT GTTGCTT GTTGCTT GTTGCTT GTTGCTT GTTGCTT GTTGCT	1210 1711BACARI 1340 ACTECHGAT ACTECHGAT 1470 CCTUTCARA CCTGTCARA CCTGTCARA CCTGTCARA CCTGTCARA CCTGTCARA CCTGTCARA CCTGTCARA	1220 1220 1350 1350 1350 1361 1350 1361 1360 1360 1360 1360 1360 1360 136	1230 RERERECTOR CRESTETT CRESTETT 1350 REFERENCE TORREST 1450 RECEGETORREST 1450 RECEGETORREST 1520 RECEGETORREST 1520 RECEGETORREST 1520 RECEGETORREST 1520	1240 LEGIACETG LEGIACETG LEGIACETG LEGIACETG LITTORNIAGE LITTORNIA	1250 GGCARTATGAT GGCARTATGAT GGCARTATGAT 1330 CONTATTATGAT 1310 CONTATTATGAT 1510 CONTATTAGAT CONTATTAGAT 1540 CONTAGAT 1640 CONTAGAT 1640	1260 1260 1360 1360 1370 1390 1390 1390 1320	1270 TTIARGERST TTARGERST TTARGERST 1400 ECTTEGIST 1530 TEGGRATTEG TEGGRATTEG TEGGRATTEG TEGGRATTEG TEGGRATTEG TEGGRATTEG	1280 HTMSCREAGHER HTMSCREAGHER HTMSCREAGHER 1410 TECCECCECE TECCECCECE 1540 GRITTHRANGG GRITTHRANGG GRITTHRANGG 1670 CTICTHRANGG CTICTHRANGG	1280 ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN ITGTTGRGGAN 1420 ECHIETRETER ECHIETRETER ECHIETRETER ECHIETRETER ARABITTTTCT ARABITTTTCT 1680	1300 INTERNITY INTERNITY 1430 INTERNITY INTERNITY 1500 INTERNITY I

Fig. 6

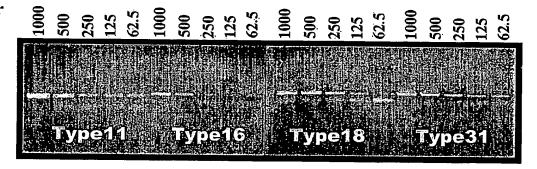
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Consensus	HECLE	I I HGGEGT	EGGE, TTRGTG	i concern	.ttoARtAA	TELERTER	acoGRARat.	ct.eTeg.tot	Teadgg. 39.	ctest.asGR	tooTAGgGa.	aaTgTatcta	Tegattara	INCRESSE INCRESSE
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HPV11-L1 HPV16-L1 HPV31-L1 HPV18-L1 Consensus	ACARC ACARC	TOTETTTA TOTETTTA	OTGGGCTGTGC ATTGGTTGCAA CTTGGTTGCAA TTGGGCTGTGC .TgGGCTGLgc	ICCRECTATA RECACCTATT CCCTGCTATT	GGGGAACACT GGGGAGCATT GGGGAACACT	regeecanne regeetanne regeetanne	GHTCCCCATG GTAGTCCTTG GCACTGCTTG	FRECONTGTTE FRETARCASTE FRANTEGEGTE	COSTORATCO CTATTACCO CTYTATCOC	AGGTGATTGT TGGTGATTGT TGGTGACAGGG	CCACCATTAG CCTCCATTAG CCCCCTTTAG	AGYTRAYARA ARTTRAAARA ARCTTRAARA	CACAGTTATA TTCAGTTATA CACAGTTATA	CREGAT
	781	790	800	810	829	830	840	850	860	870	680	890	900	910
HPV11-L1 HPV16-L1 HPV31-L1 HPV18-L1 Concensus	GGGGA	TATEGTAS TATEGTAS	ATACAGETTT ATACTOSETTT ATACTOSETTT ATACTOSATAT ATACTOSATET	IGTGCTATGG GRGCTATGG GGTGCCATGG	RETTTRETRE RETTTRETSE RETTTRETRE RETTTRELEG	TTTREAGG TTTREARGA CATTGEARGA TTaCAAge	TRACRARASTI CRCTRARASTI TRCTRARASTI CRALARALELI	INAGTTCCACT HATGTTCCTTT INAGTHCCATT INAGTLCC.OT	GGATATTTG GGACATTTG GGATATTTG	ACATETATTI ANTTETATTI CASTETATTI	GCARATATCC GTARATATCC GTARATATCC	NGATTATATY NGATTATCTY TGATTATCTY	RARATGGTGT RARATGGTTC	CAGABC CTGRGC
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Fig. 7

copy

(A)

number



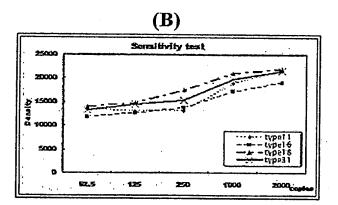


Fig. 8

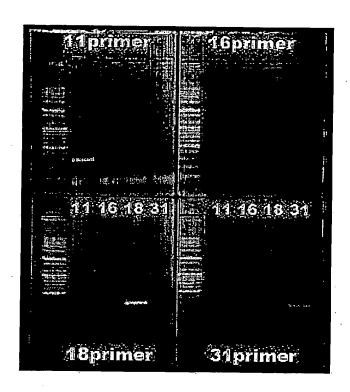
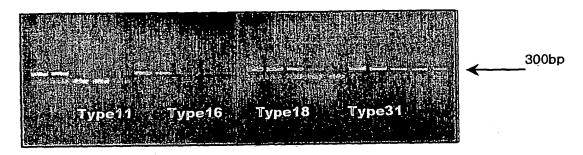


Fig. 9

a. Time point : 0 week



b. Time point: 3 week

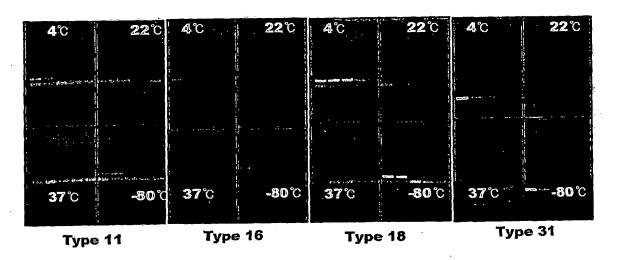
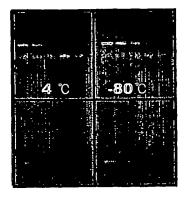


Fig. 10



Type11:4 C & -80 C

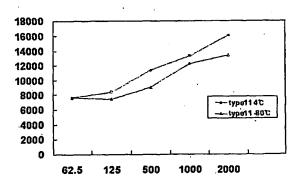
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Y=4.219X +8242.484 R=0.97 Type16:4 ℃ & -80 ℃

Y=1.888X +7370.587 R=0.99

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Type11 & Type16



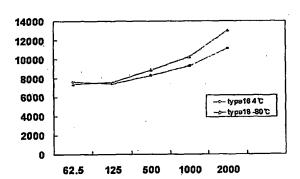
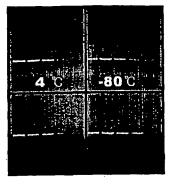


Fig. 11



Type18:4 ℃ & -80℃

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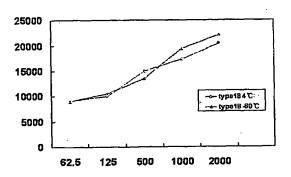
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Type31:4 C & -80 C

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Type18 & Type31



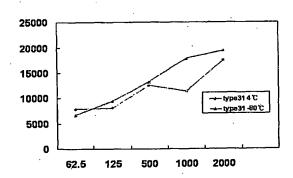
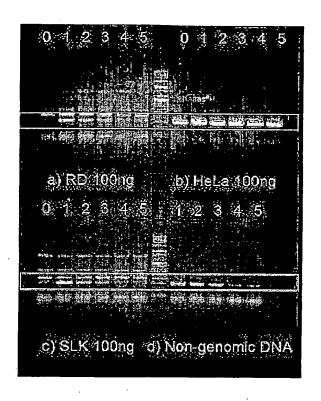


Fig. 12



WO 2006/098582

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International application No. PCT/KR2006/000915

A. CLASSIFICATION OF SUBJECT MATTER

C12Q 1/68(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC8 C12Q 1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and applications for inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)

NCBI PubMed, NCBI GenBank, eKIPASS "HPV, L1, high-risk probe, genotyping, etc."

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
KR 2004/0036318 A (GENOMICTREE, INC., KR) 30 Apr. 2004 - see the whole document	1-8
KR 2004/0078506 A (BIOMEDLAB CO., LTD., KR) 10 Sep. 2004 - see the whole document	1 - 8
WO 2001/068915 A1 (BIOMEDLAB CO., LTD., KR) 20 Sep. 2001 - see the whole document	1 - 8
WO 2003/027323 A1 (BIOMEDLAB CO., LTD., KR) 03 Apr. 2003 - see the whole document	1 - 8
KR 2004/0083674 A (BIOCORE CO., LTD., KR) 06 Oct. 2004 - see the whole document	i - 8
	KR 2004/0036318 A (GENOMICTREE, INC., KR) 30 Apr. 2004 - see the whole document KR 2004/0078506 A (BIOMEDLAB CO., LTD., KR) 10 Sep. 2004 - see the whole document WO 2001/068915 A1 (BIOMEDLAB CO., LTD., KR) 20 Sep. 2001 - see the whole document WO 2003/027323 A1 (BIOMEDLAB CO., LTD., KR) 03 Apr. 2003 - see the whole document KR 2004/0083674 A (BIOCORE CO., LTD., KR) 06 Oct. 2004

See patent family annex.

- * Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- E" earlier application or patent but published on or after the international filing date
- 'L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
- O" document referring to an oral disclosure, use, exhibition or other
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority
 date and not in conflict with the application but cited to understand
 the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of mailing of the international search report

Date of the actual completion of the international search

10 JULY 2006 (10.07.2006)

10 JULY 2006 (10.07.2006)

Authorized officer

Name and mailing address of the ISA/KR



Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

SHIN, Kyeong A

Telephone No. 82-42-481-5589



International application No.

PCT/KR2006/000915

Box No. I	. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)	
1. With re inventi	regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the ntion, the international search was carried out on the basis of:	claimed
a. typ [> [>	type of material a sequence listing table(s) related to the sequence listing	·
b. for	ormat of material on paper in electronic form	
c. tim	ime of filing/furnishing contained in the international application as filed filed together with the international application in electronic form furnished subsequently to this Authority for the purposes of search	
. — (In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has be or furnished, the required statements that the information in the subsequent or additional copies is identical to that application as filed or does not go beyond the application as filed, as appropriate, were furnished.	
3. Additi	litional comments:	
		•
•		
		•
	<u>.</u>	

International application No.

PCT/KR2006/000915

Box No. 11 Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)	_					
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:						
1. Claims Nos.: 3-7 because they relate to subject matter not required to be searched by this Authority, namely:						
Although claims 3-7 relate to a method of diagnosing under Rule 39.1(iv), the search has been carried out and based on the alleged effects of the method.						
2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:						
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).						
Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)						
This International Searching Authority found multiple inventions in this international application, as follows:						
1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.						
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.						
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:						
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claims Nos.:						
Remark on Protest The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee. The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation. No protest accompanied the payment of additional search fees.						

Information on patent family members

International application No.
PCT/KR2006/000915

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
KR 2004/0036318 A	30/04/2004	None	· · · · · · · · · · · · · · · · · · ·
KR 2004/0078506 A	10/09/2004	None ·	
WO 2001/068915 A1	20/09/2001	AU 200110604 A1 CN 1350593 A EP 1263984 A1 JP 15527586 A KR 2001091450 A	24/09/2001 22/05/2002 11/12/2002 16/09/2003 23/10/2001
WO 2003/027323 A1	03/04/2003	CN 1558954 A EP 1434873 A1 JP 17503177 A KR 2003027178 A US 20040265794 A1	29/12/2004 07/07/2004 03/02/2005 07/04/2003 30/12/2004
· KR 2004/0083674 Å	06/10/2004	None	